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Validation of Sixteen Leukemia and Lymphoma Cell Lines as Controls for Molecular Gene Rearrangement Assays

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Background: **Assays for rearrangement of the immunoglobulin, T-cell receptor, *bcr/abl*, and *bcl-2* genes are valuable tools to aid in the diagnosis of leukemias and lymphomas and are now offered by many pathology laboratories. However, there is a lack of well-characterized and validated calibrators and positive controls for these assays. We therefore evaluated 16 readily available leukemia and lymphoma cell lines for their potential use as controls.**

Methods: **DNA and RNA were isolated from each cell line and analyzed by Southern blot and PCR or reverse transcription-PCR (RT-PCR). Rearrangements in the IgJ_H, IgJ_w, *TcR-β* or *TcR-γ*, *bcr/abl*, and *bcl-2* genes were detected by commercially available probes and primers. Cell lineages were confirmed by immunophenotyping.**

Results: **Immunoglobulin and T-cell receptor gene rearrangements were identified in five B- and three T-cell lines, respectively. Two cell lines tested positive for the *bcr/abl* gene, and one was positive for the *bcl-2* gene rearrangement by Southern blot.**

Conclusions: **The 16 cell lines studied can be used as positive controls in molecular detection assays for gene rearrangements. The parallel processing of these cell lines with clinical samples can serve to quality control the experimental procedures from the first step of DNA or RNA extraction to the final step of result analysis.**

Advances in molecular biology have created powerful and useful techniques that complement current clinical laboratory and pathology testing within many disciplines, including genetics, oncology, infectious diseases, and blood transfusion *(1–4)*. During the past decade, assays based on Southern blot and PCR *(5–7)* have been applied to oncology, where molecular genetic analysis enables the detection of chromosomal abnormalities to study the correlation of gene rearrangements with standard pathologic evaluation and tumor development *(8, 9)*.

Clinical molecular biology laboratories commonly use molecular analyses to determine clonality of B *(10–12)* and T cells *(13, 14)* in leukemia and malignant lymphoma *(5, 15, 16)* and to detect the translocations t(14;18) in follicular lymphoma *(17–19)* and t(9;22) in chronic myelogenous leukemia *(20–22)*. In the future, new analytical methods may provide even broader clinical applications of molecular detection in pathologic diagnosis and evaluation *(23–26)*.

The detection of specific gene rearrangements adds important information to the evaluation and diagnosis of patients and supplements results from cytogenetics and immunophenotyping. However, the detection of clonality and/or gene rearrangements by molecular techniques depends on the visualization of DNA fragments different from those seen in nonmalignant germline DNA and which frequently represent unique molecular events in a small percentage of cells in the total cell population *(13, 14, 27)*. The overall performance and quality of the assay are assessed with control samples that are analyzed in parallel with the patient samples and most commonly consist of previously tested and validated patient specimens. However, the limited amount of such material requires frequent characterization and validation of new positive control samples when the old ones run out. Furthermore, because these samples are generally stored as DNA, they do not allow controlling for the initial steps of DNA or RNA isolation. A readily available and renewable source of cells would greatly simplify some of these issues and promote the universal standardization of these assays.

In this report, we establish the utility of leukemia and lymphoma cell lines as positive controls in molecular gene rearrangement assays. Their gene rearrangement patterns were defined by Southern blot analysis and PCR or reverse transcription-PCR (RT-PCR) using commercially

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available probes or primers for the immunoglobulin genes IgJ_H and IgJ_κ for B-cell origin, the T-cell receptor genes *TcR-β* and *TcR-γ* for T-cell origin, and *bcl-2* for the t(14;18) and *bcr/abl* for the t(9;22) translocations, respectively. For comparison, the B- and T-cell origins of the cell lines were verified by immunophenotyping using flow cytometry.

Materials and Methods

CELLS AND DNA AND RNA PREPARATIONS

All cell lines were obtained from the American Tissue Culture Collection and cultured as recommended (Table 1). DNA was isolated using the Puregene DNA reagent set (Gentra), and RNA was isolated using the RNeasy reagent set (Qiagen). The DNA and RNA concentrations were determined from absorbance measurements at 260 and 280 nm.

SOUTHERN BLOT

DNA (10 μg) was digested with the appropriate restriction enzymes and the recommended buffers (Roche Molecular Biochemicals), and was separated in a 0.7% agarose gel by electrophoresis at 100 V for 3 h in Tris-borate-EDTA buffer. The DNA was transferred to nylon membranes (Roche Molecular Biochemicals) overnight by capillary action. The probes were labeled with [α-^{32}P]dCTP (NEN Life Science Products), using the prime-it II random primer labeling reagent set (Stratagene), and purified by QIAquick column purification (Qiagen). IGHJ6 and IGKJ5 probes for detection of IgJ_H and IgJ_κ gene rearrangements, respectively, were purchased from Dako. TransProbe-1, which detects the Philadelphia chromosome translocation t(9;22) was obtained from Oncogene Science Diagnostics. All control DNA samples and the *bcl-2* probe for detection of the t(14;18) translocation were purchased from Oncogene Research products. Each specific probe was hybridized to the membrane-bound DNA by use of Quick hybridization solution (Stratagene) according to the manufacturer's recommendations. The membrane was then exposed to a phosphorimager screen for detection of bound radiolabeled probe. The fluorescein-labeled TCRBC probe (Dako) was used to detect the *TcR-β* gene rearrangement, using the Dako chemiluminescent system with exposure to x-ray film (Kodak) according to the manufacturer's instructions.

RT-PCR AND PCR

cDNA was synthesized with Omniscript reverse transcriptase using 1 μg of RNA and random hexamer primers (Qiagen). PCR was performed with gene-specific PCR master mixtures containing the appropriate primer pairs as supplied by In Vivo Scribe. The following reagent sets were used: cat. no. 105G-24 for *bcl-2* gene rearrangement; cat. no. 102G-24 for *IgH* and *TcR-γ* gene rearrangements; cat. no. 103G-24 for *bcr/abl* t(9:22) translocations; and cat. no. 107G-24 for immunoglobulin κ light chain assays. The primers are an integral part of the master mixture in each reagent set, but their sequences are proprietary to In Vivo Scribe and therefore not given in the instructions that accompany the reagent sets. Either 500 ng of DNA or 5 μL of cDNA from the reverse transcription reaction were used as the template and amplified at 94 °C for 45 s (3 min for the first cycle), 55 °C for 45 s, and 72 °C for 1 min (10 min for the last cycle) for 35 cycles. Nested PCR was performed with 5 μL of a 1:100 (for *bcl-2*) or 1:10 (for *bcr/abl*) dilution of the first reaction as template. The PCR products were analyzed on a 2% agarose gel at 100 V for 2 h.

IMMUNOPHENOTYPING

The cultured cells were washed twice in Dulbecco's phosphate-buffered saline, pH 7.1, containing 1 g/L NaN_3 and suspended at a density of 5×10^6 cells/mL in this buffer. One hundred-microliter aliquots of the cells were then added to individual 12 × 75 mm Falcon 2052 plastic tubes for incubation with fluorescence-labeled antibodies to detect surface cluster of differentiation (CD) markers. The fluorescence-labeled antibodies used in this study were from Becton Dickinson (CDs 3, 4, 8, 19, and 20), Beckman Coulter (CDs 2, 7, TcRαβ, TcRγδ, 10, and 11c), and Biosource International (CDs sκ, sλ, IgD, and IgM). They comprised markers specific for T cells (CDs 2, 3, 4, 7, 8, TcRαβ, and TcRγδ) and B cells (CDs 10, 11c, 19, 20, sκ, sλ, IgD, and IgM). Stained cells were washed once in phosphate-buffered saline, suspended, and fixed in 500 μL of phosphate-buffered saline containing 5 mL/L formaldehyde. Fluorescence was detected on a FACScan flow cytometer (Becton Dickinson), and acquired and analyzed using CELLQuest software (Becton Dickinson) run on a Power Macintosh Model 7600/132 (Apple). Negative controls consisted of fluorescence-labeled nonspecific antibodies of the same isotype as the CD-specific antibodies.

Results

DETECTION OF B- OR T-CELL LINEAGE

To identify cell lines that represent the B- or T-cell lineage, DNA samples from 13 known B- and T-cell lines, together with control samples, were digested with the restriction enzymes *Eco*RI, *Bam*HI/*Hin*dIII, and *Xba*I and examined by Southern blot using labeled IGHJ6, IGKJ5, and TCRBC probes (Table 1). The 1020-bp IGHJ6 probe detects rearrangements in the J_H segments of the immunoglobulin heavy-chain gene (Fig. 1A). Compared with placental DNA, which represents the germline configuration (Fig. 1A, lane 13), *Eco*RI digestion of DNA extracted from RL, Nam, SB, and Raji cells generated novel bands of distinct sizes (Fig. 1A), whereas the typical 8-kb germline band disappeared in DNA from Daudi cells (Fig. 1A, lane 5). *Bam*HI/*Hin*dIII digestion of DNA from D1, RL, Jcam, Nam, Daudi, SB, J45, Jurkat, and Raji cells and *Xba*I digestion of RL, Nam, Daudi, and SB cells generated various and distinct novel bands, whereas Raji lost the typical *Xba*I germline band (Fig. 1A, lane 11). When

Table 1. Summary of the Southern blot analyses using J_H probe IGHJ6, Jκ probe IGKJ5, *TcR-β* probe TCRBC, *bcr/abl* TransProbe-1, and *bcl-2* probe.

Cell line	IGHJ6	IGKJ5	TCRBC	TransProbe-1	*bcl-2*	ATCC[a] no.
SB	+	+	−	−	−	CCL-120
Raji	+	+	−	−	−	CCL-86
Nam	+	+	−	−	−	CRL-1432
Daudi	+	+	−	−	−	CCL-213
SupB15	NA	NA	−	−	NA	CRL-1929
RL	+	+	−	−	+	CRL-2261
D1	−	−	+	−	−	CRL-10915
Molt3	−	−	+	−	−	CRL-1552
Jurkat	−	−	−	−	−	TIB-152
CEM	−	−	−	NA	−	CCL-119
Jcam	−	−	−	−	−	CRL-2063
J45	−	−	−	−	−	CRL-1990
Sup T1	NA	NA	+	NA	NA	CRL-1942
K562	NA	NA	NA	+	NA	CCL-243
MEG	NA	NA	NA	+	NA	CRL-2021
HL60	−	−	NA	−	+	CCL-240

[a] ATCC, American Type Culture Collection; NA, results not available.

analyzed by PCR (Table 2), RL, SB, and Raji cells showed an ~317-bp product with the FR1 primer pair (Fig. 1B), and Nam, SB, and Raji cells showed an ~104-bp product with the FR3 primer pair (Fig. 1C), both of which demonstrated rearrangements in IgJ_H genes. Although the exact sizes are likely to be somewhat heterogeneous among different samples, discrimination of small differences was beyond the resolving power of the agarose gels used. However, all the products were near the expected sizes, as deduced from the positive control, and small differences did not change the interpretation. Several nonspecific bands were also observed with the FR1 primer pair, but they were larger than the expected product range of ~290–350 bp (Fig. 1B, lanes 1, 3, 7, 8, 9, 10, and 12). In this and the other PCR assays, extra bands were frequently obtained with some of the primer pairs provided with the In Vivo Scribe reagent set. These were generally regarded as nonspecific based on the information provided by the company and the supplied controls. However, we were unable to eliminate these bands, despite trying various reaction conditions. Whether future generations of the primers will alleviate this problem remains to be seen. Thus, it is extremely important to include positive controls that give a PCR product in the expected size range.

The 540-bp IGKJ5 probe was used to detect rearrangements in the Jκ region of the immunoglobulin κ light chain gene (Fig. 2A). Compared with placental DNA (Fig. 2A, lane 13), *Eco*RI digestion of DNA from RL, Nam, SB, and Raji cells produced distinct novel bands, whereas the typical 9.3-kb *Eco*RI germline band disappeared in Daudi cells. For the *Bam*HI/*Hind*III restriction digestion, the IGKJ5 probe revealed novel digestion patterns in RL, Nam, Daudi, SB, and Raji cells. *Xba*I digestion of DNA from Daudi, SB, and Raji cells generated various novel bands, whereas the typical 9.9-kb germline band was lost

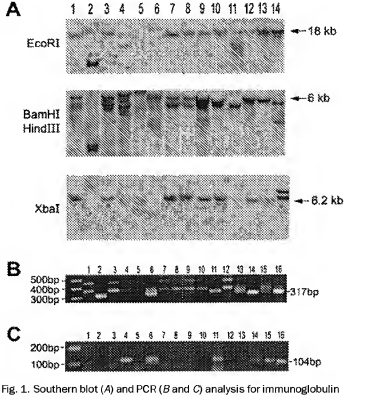

Fig. 1. Southern blot (*A*) and PCR (*B* and *C*) analysis for immunoglobulin heavy chain rearrangements.

(*A*), 10 μg of DNA from various cell lines was digested with *Eco*RI, *Bam*HI/*Hin*dIII, or *Xba*I, separated by agarose gel electrophoresis, and transferred to a nylon membrane. Each membrane was then hybridized with a probe for J_H as described in *Materials and Methods*. *Lanes*: *1*, D1; *2*, RL; *3*, Jcam; *4*, Nam; *5*, Daudi; *6*, CORF-SB; *7*, J45; *8*, Jurkat; *9*, CEM; *10*, HL60; *11*, Raji; *12*, Molt3; *13*, placental; *14*, 20% SU-DHL-4. (*B*), DNA from the same cell lines as in *A* was analyzed by PCR with primer pair FR1 as provided in the In Vivo Scribe reagent set. *Lanes 1–13*, same as in *A*; *lane 14*, 1% RL; *lane 15*, 10% control; *lane 16*, FR1 control. (*C*), lanes are the same as in *B*, except that FR3 primers were used. *Lanes 1–13*, same as *A*; *lane 14*, 20% In Vivo Scribe control; *lane 15*, 10% Nam; *lane 16*, FR3 In Vivo Scribe control.

Table 2. Summary of PCR analyses using the IgH primers FR1 and FR3; IgK primers; *TcR-γ* primers 1 and 2; *bcr/abl* primers 2b, 2c, 3b, 3d, and 3d; and *bcl-2* mbr primers.

	IgH			*TcR-γ*		*bcr/abl*					
Cell line	**FR1**	**FR3**	**IgK**	**1**	**2**	**2b**	**2c**	**3b**	**3c**	**3d**	***bcl-2***
SB	+	+	+	−	−	−	−	−	−	−	−
Raji	+	+	−	−	−	−	−	−	−	−	−
Nam	−	+	+	−	−	−	−	−	−	−	−
Daudi	−	−	−	−	−	−	−	−	−	−	−
SupB15	NA[a]	NA	NA	−	−	−	+	−	−	−	NA
RL	+	−	−	−	−	−	−	−	+	−	+
D1	−	−	−	+	+	−	−		−	−	−
Molt3	−	−	−	−	−	−	−		−	−	−
Jurkat	−	−	−	+	+	−	−		−	−	−
CEM	−	−	−	+	+	−	−		−	−	−
Jcam	−	−	−	+	+	−	−		−	−	−
J45	−	−	−	+	+	−	−		−	−	−
Sup T1	NA	NA	NA	+	+	NA	NA		−		NA
K562	NA	NA	NA	NA	NA	−	−	+	+	+	NA
MEG	NA	NA	NA	NA	NA	−	+	−	+	−	NA
HL60	−	−	−	NA	NA	−	−	−	−	−	−

[a] NA, results not available.

in DNA from RL and Nam cells. When analyzed by PCR, Nam and SB cells also showed an ~219-bp product with the Jκ primers, which represents rearrangements in the Jκ locus (Fig. 2B).

The 378-bp TCRBC probe was used to detect rearrangements in the *TcR-β* locus of the T-cell receptor genes (Fig. 3A). Compared with placental DNA, the DNA from

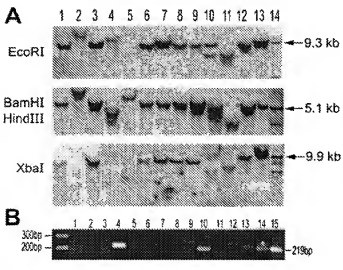

Fig. 2. Southern blot (*A*) and PCR (*B*) analysis for immunoglobulin light chain rearrangements.

(*A*), conditions were the same as described in the legend for Fig. 1, except that each membrane was hybridized with a probe for Jκ as described in *Materials and Methods*. *Lanes: 1*, D1; *2*, RL; *3*, Jcam; *4*, Nam; *5*, Daudi; *6*, J45; *7*, Jurkat; *8*, Molt3; *9*, CEM; *10*, CCRF-SB; *11*, Raji; *12*, HL60; *13*, placental; *14*, 20% SU-DHL-4. (*B*), DNA from the same cell lines as in *A* was analyzed by PCR with primer pair IgK as provided in the In Vivo Scribe reagent set. *Lanes 1–13*, same as *A*; *lane 14*, 10% In Vivo Scribe control; *lane 15*, IgK control.

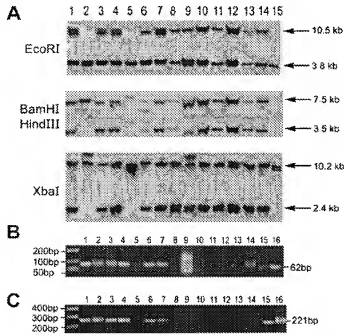

Fig. 3. Southern blot (*A*) and PCR (*B* and *C*) analysis for *TCR-β* (*A*) and *TCR-γ* gene rearrangements (*B* and *C*).

(*A*), conditions were the same as described in the legend for Fig. 1, except that each membrane was hybridized with a probe for *TcRβ* as described in *Materials and Methods*. *Lanes: 1*, J45; *2*, SupT1; *3*, Jcam; *4*, D1; *5*, Molt3; *6*, CEM; *7*, Jurkat; *8*, CCRF-SB; *9*, Nam; *10*, Raji; *11*, Daudi; *12*, RL; *13*, SupB15; *14*, placental; *15*, 20% Molt3. (*B*), DNA from the same cell lines as in *A* was analyzed by PCR with primer pair *TcR-γ1* as provided in the In Vivo Scribe reagent set. *Lanes 1–14*, same as in *A*; *lane 15*, 10% in Vivo Scribe control; *lane 16*, TcR-γ1 control. (*C*), DNA from the same cell lines as in *A* was analyzed by PCR with primer pair TcR-γ2 as provided in the In Vivo Scribe reagent set. *Lanes 1–14*, same as *A*; *lane 15*, 10% IVS control; *lane 16*, TcR-γ2 control.

J45, SupT1, Jcam, D1, Molt3, and Jurkat cells showed rearranged patterns after *Eco*RI digestion. After *Bam*HI/*Hin*dIII digestion, the TCRBC probe revealed rearranged patterns in SupT1, Molt3, and CEM cells. Furthermore, *Xba*I digestion of SupT1, D1, Molt3, and Nam DNA also produced rearranged bands. Finally, when analyzed by PCR with *TcR*-γ primer pair 1, J45, SupT1, Jcam, D1, CEM, and Jurkat cells showed bands at ~60–80 bp that were somewhat heterogeneous in size among the different cell lines but all within the expected size range indicated by the positive control. Similarly, when analyzed with *TcR*-γ primer pair 2, these cells gave an ~221-bp product, demonstrating rearrangements in *TcR*-γ genes (Fig. 3, B and C).

The differentiation lineages of the leukocyte-derived cell lines were determined from their surface membrane antigens. Using 18 different monoclonal antibodies in groups of 3 against surface markers, immunophenotyping of 15 of the cell lines showed that CCRF-SB, Raji, Nam, Daudi, SupB15, and RL cells were reactive to several B-cell surface antigen markers, whereas D1, Molt3, Jurkat, CEM, Jcam, J45, and SupT1 cells were reactive to T-cell surface antigen markers (Table 3). As expected, no such surface markers were detected on MEG and HL60 cells because of their known non-B- or -T-cell origin.

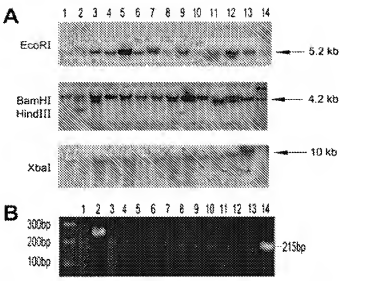

Fig. 4. Southern blot (*A*) and PCR (*B*) analysis for *bcl-2* gene rearrangement t(14;18).

(*A*), conditions were the same as those described in the legend for Fig. 1, except that each membrane was hybridized with a probe for *bcl-2* as described in *Materials and Methods*. *Lanes 1*, D1; *2*, RL; *3*, Jcam; *4*, Nam; *5*, Daudi; *6*, J45; *7*, Jurkat; *8*, Molt3; *9*, CEM; *10*, CCRF-SB; *11*, Raji; *12*, HL60; *13*, placental; *14*, 20% SU-DHL-4. (*B*), DNA from the same cell lines as in *A* was analyzed by PCR with primers for the *bcl-2* major breakpoint region as provided in the In Vivo Scribe reagent set. *Lanes 1–13*, same as *A*; *lane 14*, In Vivo Scribe mbr control.

DETECTION OF THE t(14;18) TRANSLOCATION

Twelve cell lines were examined for the t(14;18) chromosome rearrangement, using a probe that detects the *bcl-2* major breakpoint region (Fig. 4A). *Eco*RI digestion of RL, Raji, and HL60 DNA revealed distinct novel bands compared with placental DNA (Fig. 4A, lane 13). Novel bands were also detected after *Bam*HI/*Hin*dIII and *Xba*I digestion of DNA from RL cells. RL was the only cell line that was positive by PCR with the mbr primer pair (Fig. 4B).

DETECTION OF THE t(9;22) TRANSLOCATION

To determine whether any of the cell lines carried the rearranged *bcr/abl* gene, DNA was digested with the enzyme *Bgl*II and probed with TransProbe-1 (Fig. 5A).

Table 3. Immunophenotyping of the various cell lines, using 18 monoclonal antibodies (in groups of 3) against surface markers.

Sample	CD8/4/3 (T/T/T)	CD10/11c/19 (early B/HC/B)	TcRαβ/γδ/CD3 (T/T/T)	sλ/sκ/CD19 (B/B/B)	sIgD/sIgM/CD20 (B/B/B)	CD2/7/3 (T/T/T)	Cell type
SB	−/−/−	−/−/+	−/−/−	−/+wk/+	−/+wk/+	−/−/−	B
Raji	−/−/−	+/−/+	−/−/−	−/−/+	−/−/+	−/−/−	B
Nam	−/−/−	+/−/+	−/−/−	+/−/+	+/+/−	−/−/−	B
Daudi	−/−/−	+/−/+	−/−/−	−/+/+	−/+/+	−/−/−	B
SupB15	−/−/−	+/−/+	−/−/−	−/−/+	−/+/−	−/−/−	B
RL	−/−/−	+/−/+	−/−/−	+/−/+	−/+/+	−/−/−	B
D1	−/−/+	−/−/−	+/−/+	−/−/−	−/−/−	−/+/+	T
Molt3	+wk/−/−	−/−/−	−/−/−	−/−/−	−/−/−	+/+/−	T
Jurkat	+wk/−/+	−/−/−	+/−/+	−/−/−	−/−/−	+/+/+	T
CEM	+/+/−	−/−/−	−/−/−	−/−/−	−/−/−	+/+/−	T
Jcam	−/−/+	−/−/−	+/−/+	−/−/−	−/−/−	+/+/+	T
J45	+wk/−/+	−/−/−	+/−/+	−/−/−	−/−/−	+/+/+wk	T
SupT1	+/+/−	−/−/−	−/−/−	−/−/−	−/−/−	+/+wk/−	T
MEG[b]	−/−/−	−/−/−	−/−/−	−/−/−	−/−/−	−/−/−	ND
HL60[c]	−/−/−	−/+wk/−	−/−/−	−/−/−	−/−/−	−/−/−	ND

[a] +wk, weakly positive staining; ND, not detected.

[b] MEG are megakaryoblastic cells and possess no markers for B or T lymphocytes or for myeloid cells.

[c] HL60 are promyelocytic cells and possess no markers for B or T lymphocytes.

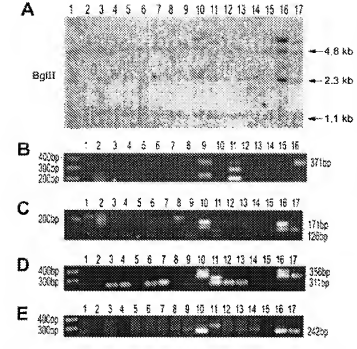

Fig. 5. Southern blot (*A*) and RT-PCR (*B–E*) analysis for the *bcr/abl* gene.

(*A*), 10 μg of DNA from various cell lines was digested with *BglII*, separated by agarose gel electrophoresis, and transferred to a nylon membrane. Each membrane was then hybridized with TransProbe-1 for *bcr/abl* as described in *Materials and Methods*. *Lanes*: *1*, D1; *2*, RL; *3*, Jcam; *4*, Nam; *5*, Daudi; *6*, J45; *7*, Jurkat; *8*, Molt3; *9*, SupB15; *10*, K562; *11*, MEG; *12*, CCRF-SB; *13*, Raji; *14*, HL60; *15*, placenta; *16*, oncogene control K562; *17*, 20% oncogene K562. (*B*), RNA from the same cells as in *A* was analyzed by RT-PCR with primers for the *bcr/abl* gene product p190 as provided in the In Vivo Scribe reagent set. *Lanes 1–15*, same as *A*; *lane 16*, In Vivo Scribe p190 control. (*C–E*), conditions were the same as for *B*, except that primers for the *bcr/abl* gene product p210 were used: (*C*), primer pair 3b; (*D*), primer pair 3c; (*E*), primer pair 3d. *Lanes 1–15*, same as *A*; *lane 16*, In Vivo Scribe p210 control; *lane 17*, 10^{-4} p210 controls.

This probe spans the 5.8-kb major breakpoint cluster region in the *bcr* gene, thereby detecting the chromosomal rearrangement that produces the chimeric *bcr/abl* gene seen in chronic myelogenous leukemia patients. Among 15 cell lines tested, 2 cell lines, K562 and MEG, showed rearranged bands after *BglII* digestion (Fig. 5A, lanes 10 and 11). K562 cells showed an extra band in addition to the three germline bands (Fig. 5A, lane 10), whereas MEG cells showed one extra band and lost the 4.8-kb germline band (lane 11). When RNA was analyzed by RT-PCR, K562 cells showed 171- and 126-bp bands with primer pair 3b (Fig. 5C, lane 10), 356- and 311-bp bands with primer pair 3c (Fig. 5D, lane 10), and a 242-bp product with primer pair 3d (Fig. 5E, lane 10). MEG cells showed a 300-bp and one smaller band with primer pair 3c (Fig. 5D, lane 11), and SupB15 and MEG cells both showed a 371-bp band with primer pair 2c (Fig. 5B, lanes 9 and 11, the weakest of the three bands for MEG). Primer pairs 3b, 3c, and 3d are specific for rearrangements involving the major breakpoint cluster region that produces the p210 gene product, whereas primer pairs 2b and 2c are specific for the rearrangement that produces the p190 gene product. However, no product at the expected size was obtained with primer pair 2b in any of the cell lines tested (data not shown). Several nonspecific bands were observed, especially with primer pair 3c, but they were smaller than expected for the real product (<300 bp; Fig. 5D, lanes 3, 4, 6, 7, 12, and 13).

Discussion

The aim of this study was to evaluate commercially available cell lines for their usefulness as positive controls in assays for B- or T-cell clonality and for t(9;22) and t(14;18) chromosomal translocations. Cell lines were considered positive for a specific rearrangement if different restriction patterns were observed with at least two enzymes when compared with the germline control or if more than one novel band was observed in a single restriction enzyme digestion. By these criteria, SB, Raji, Nam, Daudi, and RL cells were positive for immunoglobulin gene rearrangements with both B-cell-specific J_H and Jκ probes (Table 1), which are widely used in the detection of a monoclonal population of B lymphocytes associated with B-cell malignant lymphoma *(28, 29)*. Their B-cell origin was further confirmed by immunophenotyping using six combinations of three different monoclonal antibodies, each against either B- or T-cell surface markers (Table 3). These B-cell lines, therefore, qualify as positive controls in assays to detect IgJ_H and/or IgJ_κ locus rearrangements associated with B-cell leukemia and lymphoma. When we used similar criteria, the D1, Molt3, and SupT1 cell lines tested positive with the T-cell-specific probe TcR-β *(14, 30)* (Table 1), and their T-cell origins were confirmed by immunophenotyping. These cell lines therefore qualify as positive controls for detection of the rearranged *TcR-β* gene.

Because PCR-based assays have become the methods of choice for initial analysis, we also analyzed each cell line by this method. For the B-cell analysis, there was an 80% correlation (four of five cell lines; Table 2) between PCR using the FR1 and/or FR3 primers and Southern blot analysis, and 40% using the IgK primers (two of five cell lines; Table 2). For T-cell analysis, three cell lines were positive by Southern blot for *TcR-β* and six cell lines were positive by PCR for *TcR-γ*. Of those, only two, D1 and SupT1, were positive by both assays. These discrepancies were likely attributable to different regions of the IgH and IgK genes and the distinctive *TcR-β* and *TcR-γ* genes being targeted by the respective primers *(31)* (In Vivo Scribe) and probes (Dako) *(28, 29, 32)*.

Although a positive control for detecting the *bcl-2* gene rearrangement, SU-DHL-4, is commercially available as DNA, we have been unable to obtain the cell line. One of the tested cell lines, RL, was positive with both the *bcl-2* probe by Southern blot analysis and the mbr primers by PCR, exhibiting the t(14;18) translocation commonly present in follicular lymphomas *(17–19)*. Thus, RL appears to be the only cell line readily available as a positive

control cell sample for monitoring the whole procedure from DNA preparation to detection.

Both K562 and MEG cells are useful as controls for the Philadelphia chromosome translocation because they tested positive with the *bcr/abl*-specific TransProbe-1 and showed the presence of the *bcr/abl* gene product p210 *(20, 21)* by RT-PCR with primer pairs 3b, 3c, and 3d (all three for K562, and only 3c for MEG). With primer pair 2c, MEG and SupB15 cells were both positive for the *bcr/abl* gene product p190 and therefore can be used as controls for detection of the p190 gene product.

Over the last several years, novel rearrangements have been reported for the *bcl-2 (33)* and *LAZ3 (BCL6)* genes *(34)* in patients with follicular lymphoma, unusual *bcr/abl* fusion transcripts (e8/a2 and e13/a2) have been reported in patients with Philadelphia chromosome-positive leukemia *(35)*, and B- or T-cell gene rearrangements have been reported in patients with intraocular lymphoma *(36)*. In addition, the application of molecular detection has led to the identification of the t(14;18) translocation in nonlymphoid tissues *(37)*. Furthermore, new technologies, such as nonradioactive PCR-single strand conformational polymorphism analysis and PCR-temporal temperature gradient gel electrophoresis for the detection of T-cell receptor γ-chain gene rearrangements *(38, 39)* and real-time quantitative PCR for demonstrating B-cell chronic lymphatic leukemia *(40, 41)* have been developed.

In conclusion, our study validated 16 cell lines as positive controls for commonly used tests for B- and T-cell clonality and for t(9;22) and t(14;18) translocations (Tables 1 and 2). The commercial availability of the positive cell lines for each test described in this study provides easily accessible routine controls to monitor and evaluate all steps in the respective assays. They may also be useful when new or updated technologies and/or test procedures are developed. In particular, their use could facilitate interlaboratory standardization and proficiency testing for this increasingly important area of clinical analysis. Finally, the ready availability of these cells can further be exploited to determine the analytical sensitivity of a procedure by mixing a "positive" cell line with a "neutral" cell line in various proportions to create dilution series.

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